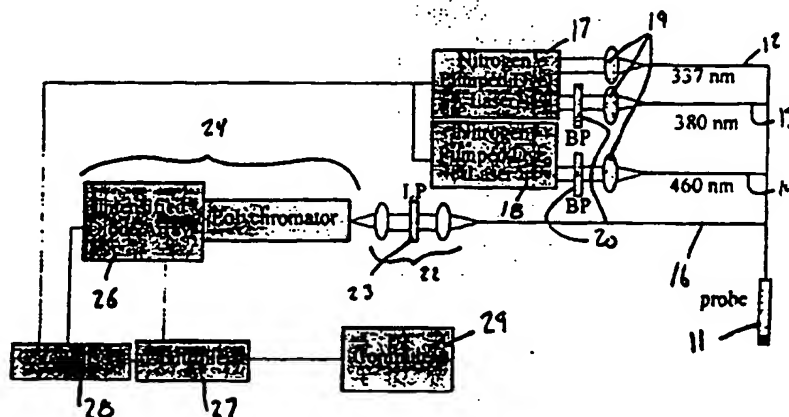


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(54) Title: METHOD AND APPARATUS FOR THE CHARACTERIZATION OF TISSUE OF EPITHELIAL LINED VISCUS



(57) Abstract

A method and apparatus for characterizing tissue of epithelial lined viscus in vivo including, for example, the endocervical canal. The method comprises illuminating an interior surface of the viscus with electromagnetic radiation wavelengths to produce a plurality of fluorescence intensity spectra, detecting a plurality of emission wavelengths from the fluorescence intensity spectra, and characterizing the epithelial viscus tissue as a function of the emission wavelengths. The apparatus includes a light source (17, 18) of emitting a plurality of electromagnetic radiation wavelengths, an optical probe (11) connected to the light source, the probe (11) being adapted to apply the plurality of electromagnetic radiation wavelengths to an interior surface of epithelial viscus tissue under test and to gather fluorescence emitted from the tissue, a detector (24, 26) connected to the probe for detecting at least one fluorescence spectrum emitted from the tissue under test and a programmed computer (29) connected to the detector for processing the at least one fluorescence spectrum according to a predetermined algorithm to characterize the tissue under test.

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DESCRIPTION**METHOD AND APPARATUS FOR THE CHARACTERIZATION OF TISSUE OF
EPITHELIAL LINED VISCUS**

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BACKGROUND OF THE INVENTION

The present invention relates to apparatus and methods for investigating epithelial lined viscus, and more particularly to apparatus and methods for characterizing normal and dysplastic tissue of the endocervical canal.

The most prevalent of preinvasive conditions of the female lower genital tract is cervical intraepithelial neoplasia (CIN). The traditional definition calls it a spectrum of intraepithelial changes that begins as a generally well differentiated intraepithelial neoplasm, which has traditionally been classified as a very mild dysplasia, and ends with invasive carcinoma. Neoplastic changes are confined to the squamous epithelium and include nuclear pleomorphism, loss of polarity, and presence of abnormal mitoses. CIN is graded 1 to 3, based on the amount of undifferentiated cells present from the basement membrane to the surface epithelium. When one third of that distance is involved, the grade is 1; when more than one third and up to two thirds is involved, the grade is 2; when more than two thirds is involved, the grade is 3. Full-thickness involvement from the surface epithelium to the basement membrane is referred to as carcinoma in situ (CIS). The median transit time from CIN to CIS depends on the grade of CIN: for grade 1 CIN, the time is approximately 6 years; for grade 2 CIN, approximately 2 years; and for grade 3, approximately 1 year. Despite some debate in the past about CIN and CIS representing two distinct entities, it is currently believed that CIN and CIS are part of a spectrum of disease that leads to invasive cancer of the cervix. The diagnosis and treatment of CIN are thus part of the prevention of invasive cervical cancer. An accepted method to classify cervical tissues is the new Bethesda system as presented in Wright *et al.*, "Pathology of the Female Genital Tract," 156-177, Springer-Verlag, (1994). In accordance with that system, lesions with HPV and CIN are classified as squamous intraepithelial lesions

(SILs) where they may be further separated as high grade SIL (CIN II, CIN III, CIS) and low grade SIL (CIN I, HPV). Normal, metaplastic and non-specific inflammation tissues are classified as non-SILs.

5 Cervical intraepithelial neoplasia is usually detected by screening Pap smears from asymptomatic women. Patients with abnormal Pap smears are referred for colposcopy and possibly biopsy. Acetic acid is applied to the cervix, and areas with abnormal DNA content, such as those with CIN, turn white. The colposcope, a mounted magnifying lens, is used to direct biopsies of the abnormal white areas. Abnormal configurations of blood
10 vessels, called vascular atypia, signal disordered growth and help the clinician know which other areas require biopsy. An appropriate evaluation of the abnormal Pap smear involves review of the referral and repeat Pap smears, endocervical curettage, and multiple biopsies of the aceto white areas; the results of such analysis will indicate whether the patient has CIN.

15 While the predictive accuracy of colposcopy is a matter of debate in the field with some researchers finding excellent overall accuracy with others finding accuracy to be poor for CIN but good for condyloma.

20 Recently, there has been intensive research to explore the use of optical spectroscopy for the diagnosis of disease in human tissue. Several studies have successfully demonstrated the use of fluorescence, infrared absorption and Raman spectroscopies for disease diagnosis in various organ systems. Auto and dye induced
25 fluorescence have shown promise in recognizing atherosclerosis and various types of cancers. Many groups have utilized autofluorescence for differentiation of normal and abnormal tissues from the human breast and lung, urinary bladder and gastrointestinal tract.

30 Copen ding Application Serial No. 08/666,021, filed July 19, 1996, assigned to the same assignee as the present application, discloses a system that uses fluorescence spectroscopy to discriminate diseased (pre-cancerous and cancerous) from non diseased

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(normal tissues and inflammation) tissue as well as differentiate cancer and high grade precancers from low grade precancerous lesions of the human cervix in vivo. This system provides more effective patient management, as 1) fluorescence measurements, and hence diagnostic information, can be obtained in real time and 2) the technique is non-invasive.

5 In vitro studies in which fluorescence was measured from cervical biopsies over the UV and visible regions of the spectrum have shown that the fluorescence intensity of histologically abnormal cervix is significantly lower than that of the normal cervix from the same patient. In accordance with the above-referenced copending application, the system includes a fiber optic probe, illumination source and optical multi channel
10 analyzer. The probe is inserted through the vaginal canal until its tip is flush with the surface of the cervix. The probe delivers light at specific excitation wavelengths and collects fluorescence from the entire emission wavelength range from a predetermined area of the cervix. During colposcopy, spectra are collected from each colposcopically abnormal area of the cervix prior to biopsy and from 1 to 4 colposcopically normal areas.
15 Using this system, laser induced fluorescence acquired from human cervical tissues in vivo at 337, 380 and 460 nm excitation is analyzed to identify cervical intraepithelial neoplasia (CIN).

A limitation of previous colposcopic and fluorescence spectroscopic systems is that
20 they are not capable of sampling the endocervix. It is known that atypical colposcopic tissue patterns occur with some frequency at the transformation zone between the squamous and columnar epithelium in the endocervical canal. See, Burke L, Antonioli DA and Ducatman BS. Colposcopy. Text and Atlas, pp. 47, 48, 61 and 62, Appleton and Large, Norwalk CT (1991) This transformation zone (also known as the squamocolumnar
25 junction) is often located well within the endocervical canal and is not easily subjected to colposcopy or fluorescence spectroscopy using existing systems which are intended primarily to assess the ectocervix. In addition, cervical lesions that exist on the ectocervix often extend into the endocervical canal, and characterization of the lesion within the endocervical canal is often an important matter.

It would therefore be desirable to provide a means to subject the endocervical canal, including the transformation zone, to fluorescence spectroscopy.

SUMMARY OF THE INVENTION

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The present invention avoids the above noted drawbacks of the prior art by providing a method and apparatus for characterizing tissue of epithelial lined viscus including, for example, the endocervical canal. In particular, in accordance with a method embodying the present invention, endocervical canal tissue is characterized *in vivo*, by
10 illuminating endocervical canal tissue *in vivo* with electromagnetic radiation wavelengths to produce a plurality of fluorescence intensity spectra, detecting a plurality of emission wavelengths from the fluorescence intensity spectra, and characterizing the endocervical canal tissue as a function of the emission wavelengths. The characterizing step may distinguish squamous epithelium and columnar epithelium tissue, normal squamous and
15 abnormal tissue, normal columnar epithelium and abnormal tissue, inflamed and abnormal tissue, low grade SIL and high grade SIL tissue, or normal and high grade SIL tissue.

In addition, the illuminating and detecting steps may comprise, illuminating a substantially cylindrical area of the endocervical canal tissue, and detecting the plurality of
20 emission wavelengths from selected portions of the cylindrical area. The illuminating and detecting steps may further comprise illuminating an area of the endocervical canal in a vicinity of a single pixel, and detecting the plurality of emission wavelengths from the single pixel, and repeating the illuminating and detecting steps to substantially cover the cylindrical surface. In another embodiment, the illuminating and detecting steps may
25 further comprise illuminating a substantially ring-shaped area of the endocervical canal, detecting the plurality of emission wavelengths from the substantially ring-shaped area, and repeating the illuminating and detecting steps to substantially cover the cylindrical surface. In yet another embodiment, the illuminating and detecting steps may further
30 comprise, illuminating a substantially line-shaped area of the endocervical canal, detecting the plurality of emission wavelengths from the substantially line-shaped area,

and repeating the illuminating and detecting steps to substantially cover the cylindrical surface.

5 In addition, the electromagnetic radiation wavelengths used to practice the method of the present invention may be in the ranges of 317-357 nm, 360-400 nm and 440-480 nm.

10 In addition, an apparatus embodying the present invention for characterizing endocervical tissue, comprises, a light source for emitting a plurality of electromagnetic radiation wavelengths; a probe connected to the light source, the probe adapted to apply the plurality of electromagnetic radiation wavelengths to an interior surface of endocervical canal tissue under test and to gather fluorescence emitted from the tissue under test; a detector, connected to the probe, for detecting at least one fluorescence spectrum emitted from the tissue under test, and a programmed computer connected to the
15 detector means, for processing the at least one fluorescence spectrum according to a predetermined algorithm to characterize the tissue under test.

The light source may be a laser light source or a filtered white light source and the plurality of electromagnetic radiation wavelengths may be about 337 nm, about 380 nm
20 and about 460 nm. The probe may include excitation optical fibers for applying the plurality of electromagnetic wavelengths to an interior surface of the endocervical tissue under test, and collection optical fibers for gathering the fluorescence emitted from the endocervical tissue under test.

25 These and other features and advantages of the present invention will become apparent to those of ordinary skill in this art with reference to the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an exemplary apparatus in accordance with the present invention usable to perform the method of the present invention.

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FIG. 2 is another exemplary apparatus in accordance with the present invention usable to perform the method of the present invention.

FIGS. 3A-3F illustrate various states of the endocervical canal.

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FIGS. 4A and 4B are an exemplary single pixel probe usable in the present invention.

FIG. 5 is another exemplary embodiment of a single pixel probe usable with the present invention.

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FIGS. 6-11 are various exemplary embodiments of a ring probe useable in the present invention.

FIGS 12A and 12B are an exemplary embodiment of a line probe useable in the present invention.

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FIG. 13 is a graphical representation of a study of endocervical canal size.

FIGS. 14 and 15 are graphs showing the optical transmission and excitation emission of cervical mucus.

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FIGS. 16 and 17 are graphs showing the optical transmission and excitation emission of fluorinated ethylene-propylene (FEP).

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FIGS. 18, 19 and 20 are exemplary fluorescence spectra obtained from endocervical canal tissue.

DETAILED DESCRIPTION

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MEASUREMENT APPARATUS

Figures 1 and 2 present exemplary embodiments of the apparatus of the present invention which are useable to practice the method of the present invention.

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Referring first to Figure 1, an apparatus is disclosed using a single pixel optical probe. Exemplary embodiments of the single pixel probe are presented in more detail below with reference to Figures 4 and 5. The apparatus includes endocervical probe 11 which, as described below in more detail, incorporates a number of optical fibers including excitation fibers 12, 13 and 14 and collection fiber 16. The excitation fibers are connected to an illumination source which may be, for example, two nitrogen lasers 17,18 (LN300C, Laser Photonics) with a dye module. Other illumination sources, for example a Xenon lamp and filter wheel (disclosed in more detail with reference to Figure 2), may also be used. Other illumination sources may also be acceptable, including, for example, various types of lasers (for example, HeCd or Ag lasers) used with or without dye modules, and various types of so-called white light sources (for example, Xe, Hg, or XeHg lamps) used with filter wheels. This illumination source produces light at frequencies that have been selected for their ability to produce fluorescence in tissue that permits characterization of the tissue. For example light at approximately 337, 380 and 460 nanometers has proven useful. This light is coupled into excitation fibers 12, 13, 14. For coupling, standard Microbench components (Spindler Hoyer) and planoconvex lenses 19 were used. The light coming out of the two dye modules is bandpass filtered by bandpass filters 21 to minimize fluorescence from the dye being coupled into the excitation fibers 12, 13 and 14. Collection fiber 16 collects the fluorescence which is projected through a coupling optics 22 (for example, Microbench, magnification 50/30) into a detector 24, for example an F/3.8 spectrograph (Monospec 18, Thermo Jarrel Ash, Scientific Measurement Systems, Inc.). In the coupling optics 22, longpass filter 23 (for example, color glass filters, Schott)

block the reflected excitation light from entering the detector. The spectrograph disperses the light onto an intensified diode array 26. Exemplary diode array 26, electronics and controller 27 are manufactured by Princeton Instruments. The system also includes gate pulser 28 which is used to control the operation of lasers 17 and 18. Lasers 17 and 18 may be controlled, for example at a 30 Hz repetition rate with a 5 nanosecond pulse duration, but other repetition rates and pulse durations may also be acceptable.

The apparatus also includes programmed computer 29 which operates to energize lasers 17 and 18 and to analyze the fluorescence spectra collected by collection fiber 16 in order to characterize the tissue sample under study. Details of this control and analysis may be found in copending Application Serial No. 08/666,021, filed July 19, 1996, the disclosure of which is incorporated herein by reference.

Referring now to Figure 2, an apparatus embodying the present invention is disclosed using a multiple pixel optical probe. Exemplary embodiments of multiple pixel optical probes are presented in more detail below with reference to Figures 6-12. The apparatus includes a multiple pixel optical probe 21 which incorporates excitation optical fibers 22 and collection optical fibers 23. Excitation optical fibers 22 are connected to receive light from illumination source 24 which may be, for example, a Xenon lamp 26 in combination with a filter wheel 27. Once again, other illumination sources, including for example, the laser source disclosed with reference to Figure 1, would also be acceptable. As with the apparatus of Figure 1, illumination source 24 produces light at frequencies that have been selected for their ability to produce fluorescence in tissue that permits characterization of the tissue.

Collection fibers 23 from probe 21 are connected to detector 28 which includes, for example, an imaging spectrograph 29 (for example, a Chromex 250 IS), and a CCD array 31 (for example, a thermoelectric cooled CCD Princeton Instruments EV 578x384). The output of detector 28 is applied to computer 32 which is programmed to control illumination source 24 and to analyze the fluorescence spectra collected by collection

fibers 23 and detected by detector 28 using, for example, the analysis methods disclosed in the aforementioned copending application.

ENDOCERVICAL CANAL MORPHOLOGY

5 Referring now to Figures 3A-F, shown are simplified representations of the cross section of the os of the endocervical canal and surrounding tissue illustrating the locations of the squamous epithelium (SE), columnar epithelium (CE) and transformation zone (TZ) of the uterus at various stages of maturity and for various medical conditions. Specifically, Figure 3A shows the neonate uterus, Figure 3B shows the premenarchal
10 uterus, Figure 3C shows the menarchal uterus, Figure 3D shows the menstruating uterus, Figure 3E shows the menopausal uterus and Figure 3F shows the postmenopausal uterus. As can be seen, the transformation zone TZ can appear on the ectocervix (for example, menstruating, Figure 3D), or well within the endocervical canal (for example, postmenopausal, Figure 3F), or anywhere in between. Since the most common location
15 for CIN and metaplasia is at or near the transformation zone, it is critical that the transformation zone be imaged when conducting fluorescence spectroscopy. This is of particular importance in menopause and postmenopause because most cervical carcinomas occur at this age, and this is when the transformation zone is most deeply within the endocervical canal.

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Other general observations of the morphology of the endocervical canal are worthy of note. After the external os, which follows a funnel type opening, the endocervical canal enlarges and gets smaller again at the inner os. The uterus opens to its full size after the internal os by a small angle. The canal can be filled inside with non-neoplastic additional
25 tissue like polyps and synechia. Polyps may fill the canal. Atrophy may be present, which results in an abnormal form of the wall (missing folds). In addition, It is known that stenosis may occur after LEEP treatments.

The folds of the columnar epithelium may typically be several centimeters deep
30 with varying shapes.. For example, in one uterus that was studied after removal by hysterectomy, the folds were a maximum of 7.83 mm with a mean depth of 3.38 mm. The

5 folds were observed to have two main directions: axial and with an angle of approximately 30 degrees to the axis of the canal. The top of this pine tree-like form points outwards the canal. The folds are filled with mucus that sticks strongly to the tissue. Flushing with saline solution will not remove the mucus. A study of the fluorescence characteristics of cervical mucus are presented below with reference to Figures 14 and 15.

OPTICAL PROBES

10 Figures 4A and 4B are a single pixel probe 11 that may be used in the apparatus of Figure 1 in accordance with the present invention. Referring to Figure 4A, optical probe 11 includes a bundle of optical fibers 41 which are packed in a fluorinated ethylene-propylene (FEP) tubing 42 that is substantially transparent to visible light and that also transmits in the ultraviolet. The FEP tubing 42 containing the fibers 41 is flexibly mounted within a second tubing 43 which may also be made of FEP. The outer diameter of tubing 43 is preferably less than 2 mm, however other dimensions may be used. The outer diameter of tube 43 is determined primarily by anatomical constraints of the endocervical canal, and is discussed in more detail below with reference to Figure 13. This dimension allows the passage of the probe through an endocervical canal with a stenosis at the outer os. The fiber 41 within tubing 42 may be rotated and axially displaced within tubing 43 in order to permit the testing of several tissue sites without moving tubing 43.

20 Although FEP has proven useful for use as the material for the tubings used in the optical probes of the present invention, other materials may also be acceptable, including, for example, other plastics such as polytetrafluorethylen (PTFE), glass and quartz.

25 In the embodiment of Figure 4A and B, a short piece of a large diameter fiber 45 is used as a reflector and the end surface 47 of fiber 45 is polished with an oblique angle (for example, 40°) relative to the axis of probe 11. Reflection of light emitted by fibers 41 toward the tissue sample under study (downward in Figure 4A) and reflection of light emitted by the tissue sample back toward fibers 41 occurs because of total internal reflection. An alternative reflector may be made using an angled mirrored surface of polished metal, glass, sapphire, or the like.

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Figure 4B is a cross section through section 4B-4B of Figure 4A, and shows the configuration of fibers 41. In the exemplary embodiment there are seven fibers 41, six illumination fibers 48-53, and one collection fiber 54, however any number of fibers may be used. In the exemplary embodiment, illumination fibers 48 and 51 are used for 337 nm, fibers 49 and 52 are used for 380 nm and fibers 50 and 53 are used for 460 nm. and collection fiber 54 provides a single pixel collection for fluorescence spectroscopy. It should be noted that any combination of illumination and collection fibers may be used without departing from the scope of the invention. For example, three illumination fibers and one collection fiber may be used, three illumination fibers and three collection fibers may be used., one illumination fiber and one collection fiber may be used, one fiber used for the combined purpose of illumination and collection may be used, and so forth. Fibers 48-54 may be, for example, type SFS320/385T optical fibers, and fiber 46 may be a type SFS1500/1650N optical fiber, both available from Fiberguide Industries, however other types may also be used. The single pixel embodiment results in a single substantially elliptical measurement and illumination spot, or pixel.

The part of probe 11 that extends outside the vagina preferably has a rigid tube with markings which may be used as an aid in positioning the probe both axially and rotationally. Saline solution may be flushed through the openings 61 in tip 62 of probe 11 before or during a testing procedure.

Figure 5 is an alternative embodiment of the single-pixel probe of the present invention. Referring to Figure 5, probe 11 includes fiber bundle 41 like that of the embodiment of Figure 4A. light emitted from the end of fibers 41 is focused by lens 66 and reflected by reflecting surface 67 toward a tissue sample 68 under study. Similarly, light emitted by a tissue sample 68 is focused by lens 66, and reflected by reflective surface 67 back toward fibers 41. Other structural details remain substantially as in Figure 4A.

Referring now to Figures 6 and 7, a ring optical probe 21 is disclosed that may be used in the apparatus of Figure 2. Probe 21 includes a number of optical fibers 72 coaxially arranged in a ring shape. In one embodiment every other one of fibers 72 are used for illumination, with the remaining fibers being used for collection. Alternatively, each fiber 72 may be used for both illumination and collection. In the embodiment of Figures 10 and 11, reflection of both illuminating light and collected light is done by a metal plug 73 with a polished reflecting surface 74. Alternately, a sapphire tip 81 may be used as shown in Figure 8.. In yet another embodiment, the ends of fibers 72 may be cleaved and polished as shown in Figure 9. In the embodiment of Figure 9, every other fiber may be used as an illumination fiber with all remaining fibers being collection fibers. This would result in adjacent fibers (for example, fibers 72' and 72'') acting together to illuminate and detect from a single tissue area 77. Alternately, each of fibers 72 may have the combined function of illumination and collection. It should be noted that for the sake of clarity, the surrounding tube is not shown in the embodiment of Figure 9.

In all embodiments of the ring probe 21, light is reflected from fibers 72 toward a tissue sample located adjacent the exterior wall of probe 21 and light emitted by the tissue sample is reflected back toward fibers 72. This results in a plurality of substantially elliptical measurement and illumination spots, or pixels distributed in a ring shape.

In the ring probe 21 embodiment of Figures 6, 7 and 8, channel 76 may be included to permit the flushing of the tissue under test with saline either before or during a test... The diameter of probe 21 may be, for example, approximately 2.8 mm, however other diameters may also work.

Referring now to Figures 12A and 12B disclosed is yet another embodiment of optical probe 21 usable in the apparatus of Figure 2. The optical probe 21 of Figures 12A and 12B is a line probe. The probe 21 includes of an illuminator that serves to illuminate a tissue sample under study, and a collector that serves to collect light emitted by the tissue sample under study. The collector in the exemplary embodiment is made of 19 100 micrometer optical fibers 122 (type SFS100/110T available from Fiberguide Industries),

however, any number of optical fibers may be used. In the disclosed embodiment, The collection of the fluorescence occurs every 1.5 mm with one of the collection fibers 122. The collection fibers 122 are polished at an oblique angle relative to the longitudinal axis of the fiber 122 (for example, 40°). The ends 123 of the collection fibers 122 are
5 positioned at different axial locations along the probe as shown in Figure 12A. In the exemplary embodiment this results in a simultaneous collection every 1.5 mm along a line approximately 2.5 cm. long. The diameter of the line probe 21 of Figure 12A is approximately 3 mm, however other diameters would also be acceptable.

10 The illuminator of probe 21 in Figure 12A includes a diffuser 124. Diffuser 124 is mounted on the top of a bundle of fibers 127. Fibers 127 may be for example type SFS200 200 micrometer optical fibers available from Fiberguide Industries, however other types of fibers may be acceptable. A reflective coating 128 over 270 degrees of diffuser 124 allows a directed illumination over approximately 90 degrees of the circumference. of
15 probe 21. The diffuser 124 is available, for example, from Rare Earth Medical.

The diffuser 124 is packed in a FEP tubing 131 that is substantially transparent in the visible and also in the ultraviolet. Included within tubing 131 is the collection bundle 122, the diffuser 124 and flushing channels 132 used to carry saline to ports 133 in probe
20 21 thus permitting flushing of the tissue either before or during testing. The outer diameter of tubing 131 is preferably less than 3 mm, however, other diameters may also be acceptable. This allows the passage of the probe through most endocervical canals.

The probe is manually placed into the endocervical canal. Because of its stiffness
25 the whole probe can be pressed against the walls of the endocervical canal while still keeping a minimal bendability.

Because 100 micron fibers 122 are used for collection the size of each measured spot or pixel in the exemplary embodiment of Figure 12 A will be smaller than
30 approximately 0.5 mm. The diameter depends on the distance of the collection fiber 122 to

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the tissue. This distance may not be constant for all fibers and typically varies from approximately 0.3 to 1 mm.

5 The illumination light passes perpendicular through the collection fibers 122. Therefore the jacket of these fibers 122 should be removed. Collection fibers 122 will then act as cylindrical lenses.

Referring now to Figure 13, presented in graphical form are the results of a study of cervical size. Because the design of the probe used in the present invention depends on the canal properties the geometrical aspects of the endocervical canal were studied. A database of 362 patients at the MD Anderson Cancer Center contained measurements of the diameter of the external os. The obtained diameter is based on the size of a dilator used at MD Anderson to measure the endocervical canal prior a LEEP or LEEP cone treatment. In a following checkup visit the canal is checked again to assure no stenosis occurred. 15 This parameter was measured in another series of 22 patients with similar results.

From Figure 13 it can be seen that the endocervical canal has a mean diameter at the outer os of 5 mm. In most patients the outer os is larger than 3 mm and smaller than 7 mm. The length of the canal was estimated from a uterus removed by hysterectomy. The endocervical canal was measured to be approximately 4 cm long. These mechanical dimensions may then be considered in determining a size of the optical probes used in the present invention. For example, the study reflected in the graph of Figure 13 indicates that the optical probe should preferably be less than 3 mm in diameter if a single sized probe is to be used for all patients. Of course, probes of different sizes may also be used. 25

In addition, in order to determine the possible effects of mucus in the endocervical canal, the transmission and fluorescence of several samples of mucus was measured, and the results are presented in graphical form in Figures 14 and 15. To produce these graphs, small amounts of mucus were diluted in 10 ml of normal buffered saline solution and placed in a 1 cm pathlength. 30

As can be seen with reference to Figures 14 and 15, the strongest emission of mucus is at 340 nm emission with an excitation at 280 nm. This will not interfere with the measurements performed by the disclosed exemplary embodiments of the present invention.

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In addition, the transmission and fluorescence of FEP tubing (the presently preferred material for use as the housing for the probes of the present invention) was measured, and the results are presented in Figures 16 and 17. As can be seen with reference to Figures 16 and 17, the fluorescence of the FEP tubing is low. However the autofluorescence of the FEP tubing is about 1/10 of the tissue fluorescence at 337 nm excitation. There is a main emission peak at 400 nm with 320 nm excitation. It was determined that this contribution could be accommodated during a probe calibration procedure, discussed in more detail below.

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CLINICAL PROCEDURE

In a clinical application, the present invention has as its purpose the characterization of epithelial viscus tissue, such as, for example, tissue of the endocervical canal. In general, when applied to the characterization of endocervical tissue, the present invention has as its purposes to: a) identify lesions extending from the ectocervix into the endocervical canal; b) detect the position of the transformation zone if present inside the endocervical canal; and c) identify squamous lesions with columnar involvement inside the endocervical canal. In general, these purposes are accomplished by measuring fluorescence spectra at spatially resolved locations inside the endocervical canal over a substantially cylindrical area of the interior surface of the tissue of the canal, and using mathematical models to characterize that tissue as a function of the measured spectra.

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Before beginning a clinical procedure, the measuring apparatus should be calibrated. To calibrate the present invention (as shown, for example in Figure 1 and 2), the background signals are obtained without any excitation which reflects the dark current of the device. This background is stored and is automatically subtracted from any fluorescence measurement. Next, the autofluorescence of the probe is determined, for

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example, by placing the probe in a brown bottle containing sterile H₂O and measuring fluorescence spectra with the excitation light on. This signal is not subtracted from the tissue fluorescence, however it may be subtracted if desired. In order to confirm calibration, a standard rhodamine solution (OD 0.446725, (λ = 550 nm, 1 cm pathlength) may be measured. Based on previous clinical work, Rhodamine has been shown to have approximately twice the intensity of squamous cervical tissue fluorescence.

During spectral measurement of tissue, if improvement in the signal to noise ratio is desired, the spectra may be accumulated 100 and 200 times, respectively at 380 and 460 nm. At 337 nm 50 accumulations have proven sufficient. However, other methods to improve the signal to noise ratio may also be used. For all three wavelengths a different background subtraction file may be used with the corresponding accumulations.

During a clinical procedure, it is desired to obtain fluorescence spectra at 3 excitation wavelengths along the substantially cylindrical surface of the entire endocervical canal with a spatial resolution of approximately 1.5 mm. This may be accomplished by use of either of the apparatus of Figures 1 or 2, using any of the optical probes of Figures 4-12. During a procedure, the outer housing of the probe is placed and advanced to the internal os of the endocervical canal. Fluorescence measurement are then started. In the case of the single pixel probe (Figures 4 and 5), the single measuring pixel is advanced both axially and angularly within the housing in order to image a sufficient number of pixels over the substantially cylindrical tissue surface. When using the ring probe (Figures 6-11), the measuring ring of pixels is advanced axially in order to image a sufficient number of pixels over the substantially cylindrical tissue surface. Finally, when using the line probe (Figure 12), the measuring line of pixels is incremented angularly in order to image a sufficient number of pixels over the substantially cylindrical tissue surface. For example, when using the line probe, four individual measurements may be taken, one each at 12, 3, 6, and 9 o'clock (i.e., every 90°). This procedure takes approximately 3 minutes to complete.

Either before or during a procedure, saline solution may be flushed over the tissue in order possibly to improve measurement accuracy by removing mucus or blood or loose tissue from the measurement site.

5 In general, if the margin of the first specimen at the endocervical side is free of dysplasia or cancer and the second specimen shows no changes it may be assumed that the canal is in a normal condition. If this margin is involved with changes it may be assumed that the first 5 mm of the canal are in an abnormal state. If the margin of the endocervical specimen contains no changes it may be assumed that the margins extend no deeper than 2
10 cm. If this specimen shows abnormal cells it may be assumed that the measurements in the canal were abnormal even after 5 mm. If the second specimen is marked as metaplasia it may be assumed that the transformation zone is inside the endocervical canal. If the first specimen shows metaplasia the transformation zone is located around the os or on the ectocervix.

15 Figures 18, 19 and 20 present groups of normalized fluorescence intensity spectra obtained *in vivo* from endocervical canals of several different patients using the method and apparatus of the present invention. In particular, Figure 18 is a group of normalized fluorescence intensity spectra obtained with 337 nm excitation, Figure 19 is a group of
20 fluorescence intensity spectra obtained using 380 nm excitation, and Figure 20 is a group of normalized fluorescence intensity spectra obtained using 460 nm excitation.

 Based upon the foregoing disclosure, these and other features and advantages of the present invention will become apparent to those of ordinary skill in this art and it will
25 be appreciated that additions, deletions and changes may be made to the disclosed embodiments without departing from the scope of the invention.

REFERENCES

The following references, to the extent that they provide exemplary experimental details or other information supplementary to that set forth herein, are incorporated by reference:

1. Wright TC, Kurman RJ, and Ferenczy A in Pathology of the Female Genital Tract (eds. A. Blaustein), 156-177, Springer-Verlag, New York (1994).
2. Barron BA, Richart RM, "Statistical model of the natural history of cervical carcinoma: II. Estimates of the transition time from dysplasia to carcinoma in situ," JNCI 45: 1025-1030 (1970).
3. Burke L, Antonioli DA and Ducatman BS., Colposcopy. Text and Atlas, Appleton and Large, Norwalk CT (1991).
4. Mitchell MF, "Diagnosis and Treatment of Preinvasive Disease of the Female Lower Genital Tract" The Cancer Bulletin. 42: 71-76 (1990).
5. Reid R, Stanhope CR, Herschman BR, Crum CP, Agronow SJ, "Genital warts and Cervical cancer," Am J Obstet Gynecol, IV: 815-823 (1984).
6. Reid R, Scalzi P, "Genital Warts and Cervical Cancer," Am J Obstet Gynecol, 153(6): 611-618 (1985).
7. Barrasso R, Coupeze F, Ionesco M, DeBrux J, "Human Papilloma Viruses and Cervical Intraepithelial Neoplasia: The Role of Colposcopy," Gynecologic Oncology, 27: 197-207 (1987).
8. Alfano RR, Pradhan A and Tang CG, "Optical spectroscopic diagnosis of cancer in normal and breast tissues," J Optic Soc Am B, 6: 1015-1023 (1989).

- 5 9. Andersson ES, Johansson J, Svanberg K and Svanberg S, "Fluorescence imaging and point measurements of tissue: applications to the demarcation of malignant tumors and atherosclerotic lesions from normal tissue," *Photochem Photobiol*, 53: 807-14 (1991).
- 10 10. Richards-Kortum RR, Rava RP, Petras RE, Fitzmaurice M, Sivak MV and Feld MS, "Spectroscopic diagnosis of colonic dysplasia," *Photochem Photobiol*, 53: 777-786 (1991).
- 15 11. Rava RP, Richards-Kortum RR, Fitzmaurice M, Cothren RM, Petras RE, Sivak M and Feld MS, "Early detection of dysplasia in colon and urinary bladder tissue using laser-induced fluorescence", *Optical methods for tumor treatment and early diagnosis: mechanisms and technique*, SPIE 1426: 68-78 (1991).
- 20 12. Wong PTT, Wong RK, Caputo TA, Godwin TA and Rigas B, "Infrared spectroscopy of human cervical cells: Evidence of extensive structural changes during carcinogenesis," *Proc Natl Acad Sci USA*, 88: 10988-10992 (1991).
- 25 13. Alfano RR, Lui CH, Sha WL, Zhu HR, Akins DL, Cleary J, Prudente R and Cellmer E, "Human breast tissues studied by IR fourier transform Raman spectroscopy," *Lasers in Life Sc*, 4: 23-28 (1991).
- 30 14. Baraga JJ, Feld MS and Rava RP, "Rapid near-infrared Raman spectroscopy of human tissue with a spectrograph and CCD detector." *Appl. Spectr*, 46: 187-190 (1992).
15. Schomacker KT, Frisoli JK, Compton CC, Flotte TJ, Richter JM, Nishioka NS and Deutsch TF, "Ultraviolet laser-induced fluorescence of colonic tissue: Basic biology and diagnostic potential," *Lasers in Surg Med*, 12: 63-78 (1992).

16. Mahadevan A, Mitchell MF, Thomsen S, Silva E and Richards-Kortum RR, "A study of the fluorescence properties of normal and neoplastic human cervical tissue," Lasers Surg Med 13:647-655, (1993).
- 5 17. Ramanujam N, Mitchell MF, Mahadevan A, Thomsen S, Malpica A, Wright TC, Atkinson, N and Richards-Kortum; In Vivo Diagnosis of Cervical Intraepithelial Neoplasia Using 337 Excitation, PNAS 91:10193, (1994).
- 10 18. Ramanujam N, Mitchell MF, Mahadevan A, Thomsen S, Richards-Kortum RR, "Spectroscopic Diagnosis of Cervical Intraepithelial Neoplasia (CIN) in vivo Using Laser Induced Fluorescence Spectra at Multiple Excitation Wavelengths," Lasers Surg Med, (in press) (1996).
- 15 19. Brookner CK, Agrawal A, Trujillo EV, Mitchell MF and Richards-Kortum RR, "Relative Risk of UV-Fluorescence Spectroscopy and Endoscopy are comparable," 24th. Annual Meeting of the American Society for Photobiology, Photochem Photobiol Supp. (in press) (1996).

CLAIMS

1. A method of characterizing endocervical canal tissue *in vivo*, comprising the steps of:
 - 5 (ii) illuminating endocervical canal tissue *in vivo* with electromagnetic radiation wavelengths to produce a plurality of fluorescence intensity spectra;
 - (iii) detecting a plurality of emission wavelengths from said fluorescence intensity spectra; and
 - 10 (iv) characterizing said endocervical canal tissue as a function of said emission wavelengths.
2. The method of claim 1, wherein said characterizing step distinguishes squamous epithelium and columnar epithelium tissue.
- 15 3. The method of claim 1, said illuminating and detecting steps comprising: illuminating a substantially cylindrical area of said endocervical canal tissue; and detecting said plurality of emission wavelengths from selected portions of said cylindrical area.
- 20 4. The method of claim 3, said illuminating and detecting steps further comprising:
 - a) illuminating an area of said endocervical canal in a vicinity of a single pixel;
 - b) detecting said plurality of emission wavelengths from said single pixel;
 - and
- 25 repeating steps a) and b) to substantially cover said cylindrical surface.
5. The method of claim 3, said illuminating and detecting steps further comprising:
 - a) illuminating a substantially ring-shaped area of said endocervical canal;
 - 30 b) detecting said plurality of emission wavelengths from said substantially ring-shaped area; and

repeating steps a) and b) to substantially cover said cylindrical surface.

6. The method of claim 3, said illuminating and detecting steps further comprising:

- 5 a) illuminating a substantially line-shaped area of said endocervical canal;
 b) detecting said plurality of emission wavelengths from said substantially
 line-shaped area; and

repeating steps a) and b) to substantially cover said cylindrical surface.

7. The method of claim 1, wherein the electromagnetic radiation wavelengths are in
10 the ranges of 317-357 nm, 360-400 nm and 440-480 nm.

8. The method of claim 7, wherein the electromagnetic radiation wavelengths are about
337 nm, about 380 nm and about 460 nm.

15 9. The method of claim 1, wherein said characterizing step distinguishes normal
squamous and abnormal tissue.

10 10. The method of claim 1, wherein said characterizing step distinguishes normal
columnar epithelium and abnormal tissue.

20 11. The method of claim 1, wherein said characterizing step distinguishes inflamed and
abnormal tissue.

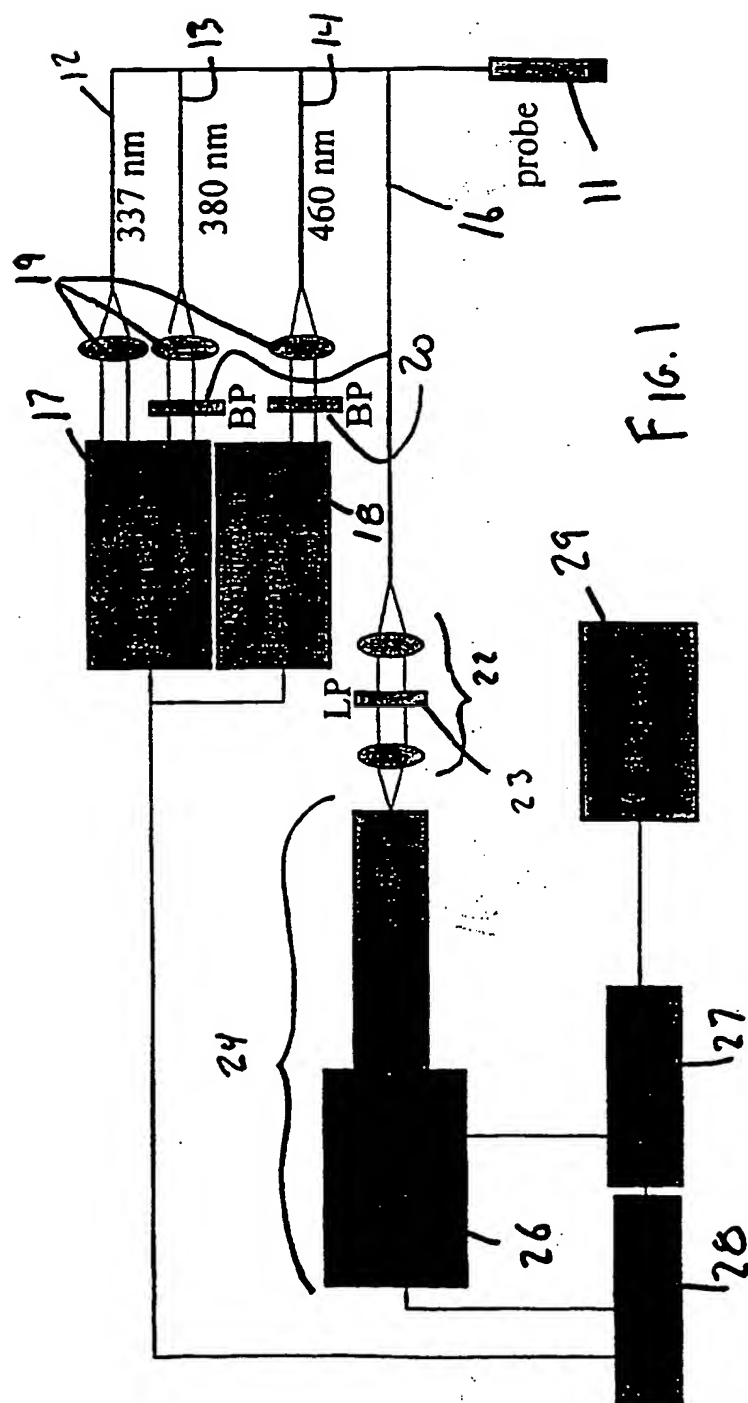
25 12. The method of claim 1, wherein said characterizing step distinguishes low grade
SIL and high grade SIL tissue.

13. The method of claim 1, wherein said characterizing step distinguishes normal and
high grade SIL tissue.

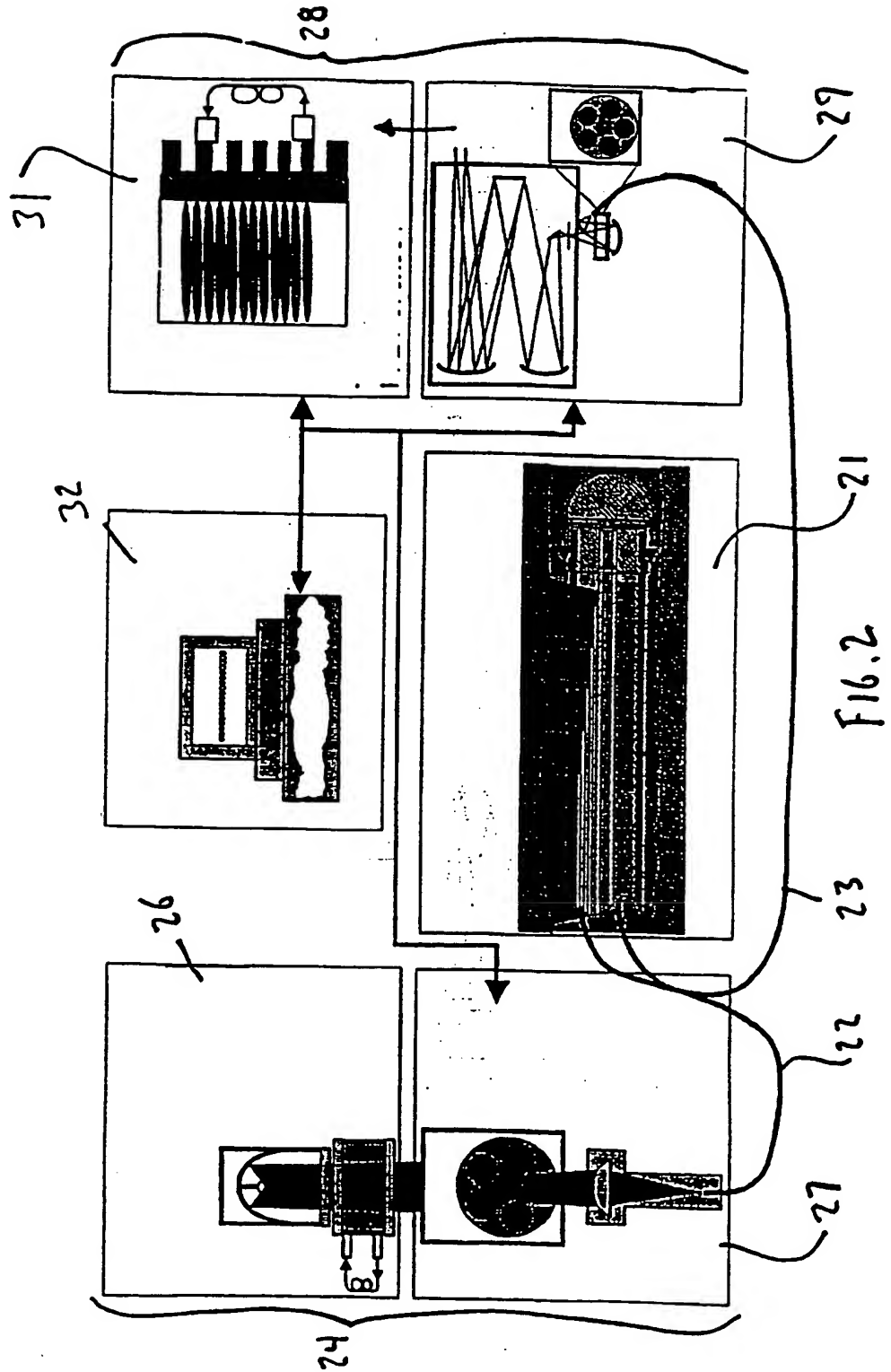
14. A method of characterizing epithelial lined viscus tissue *in vivo*, comprising the steps of:

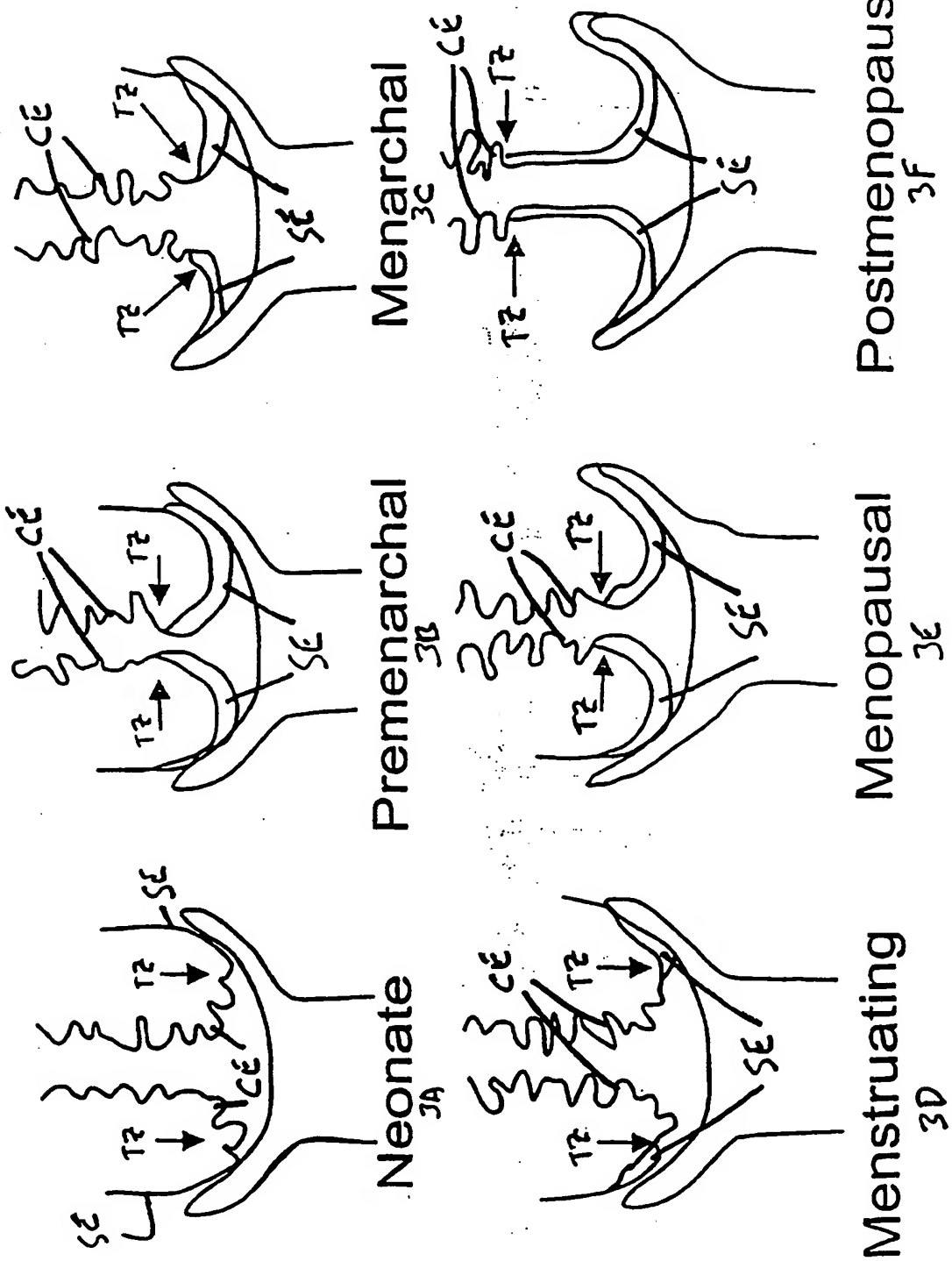
- (ii) illuminating epithelial lined viscus tissue *in vivo* with electromagnetic radiation wavelengths to produce a plurality of fluorescence intensity spectra;
- (iii) detecting a plurality of emission wavelengths from said fluorescence intensity spectra; and
- (iv) characterizing said epithelial lined viscus tissue as a function of said emission wavelengths.

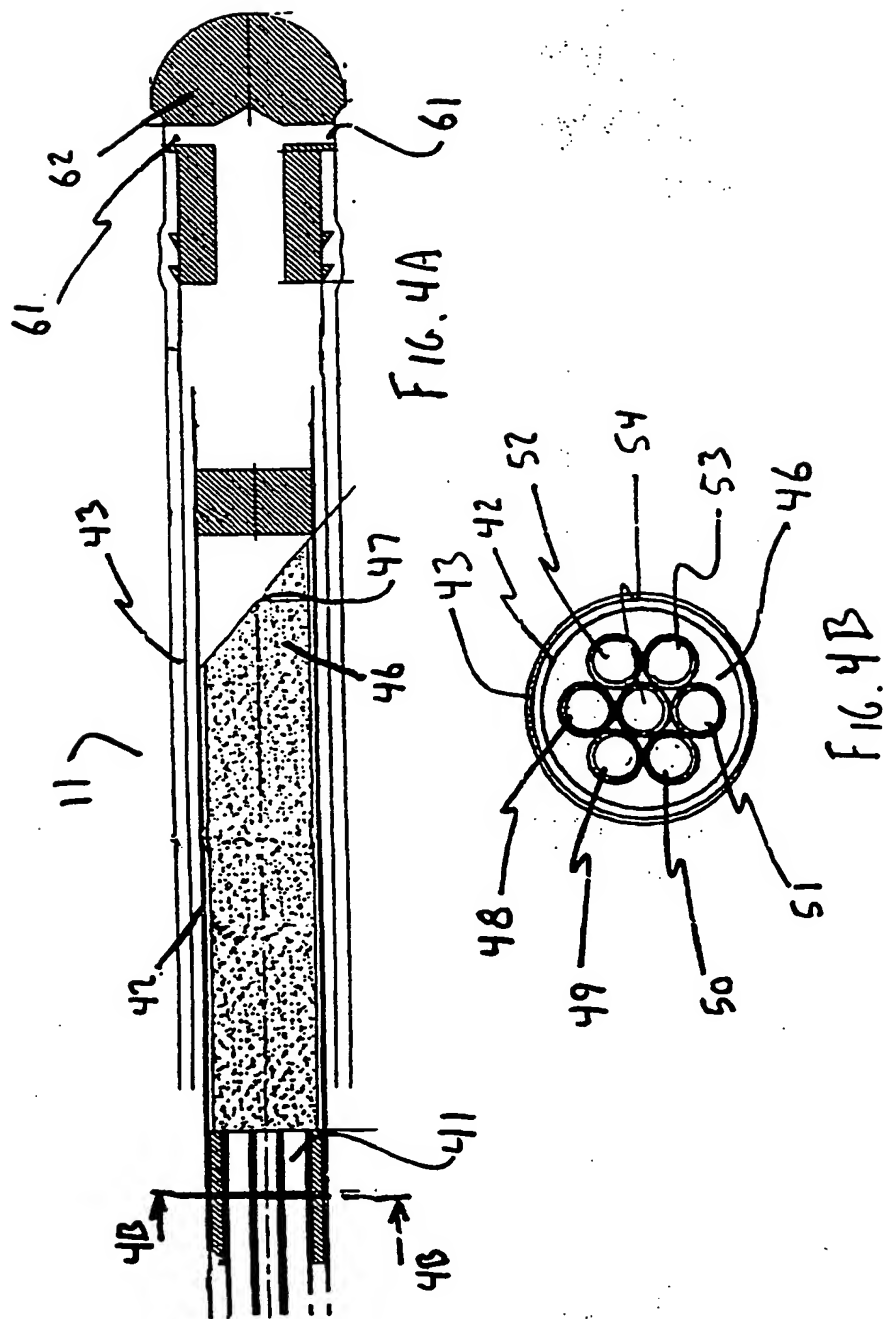
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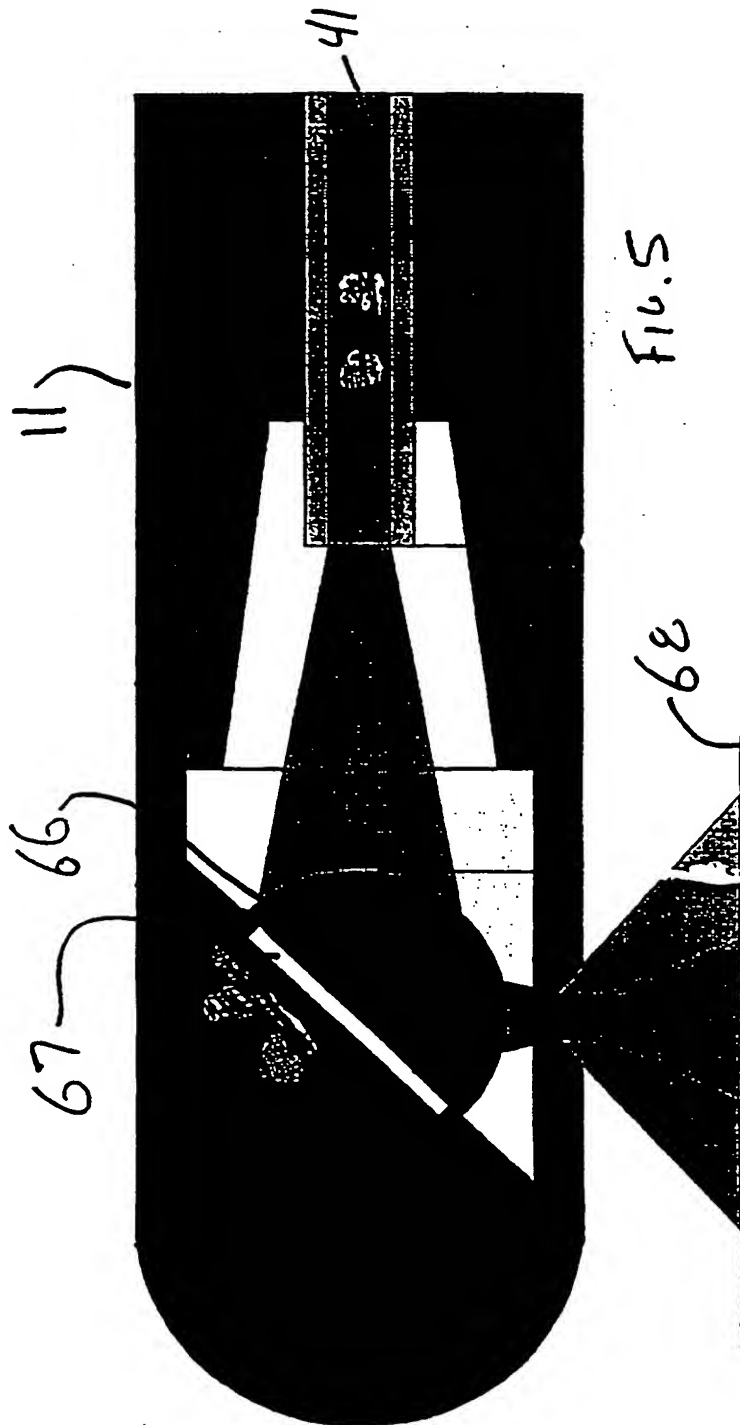
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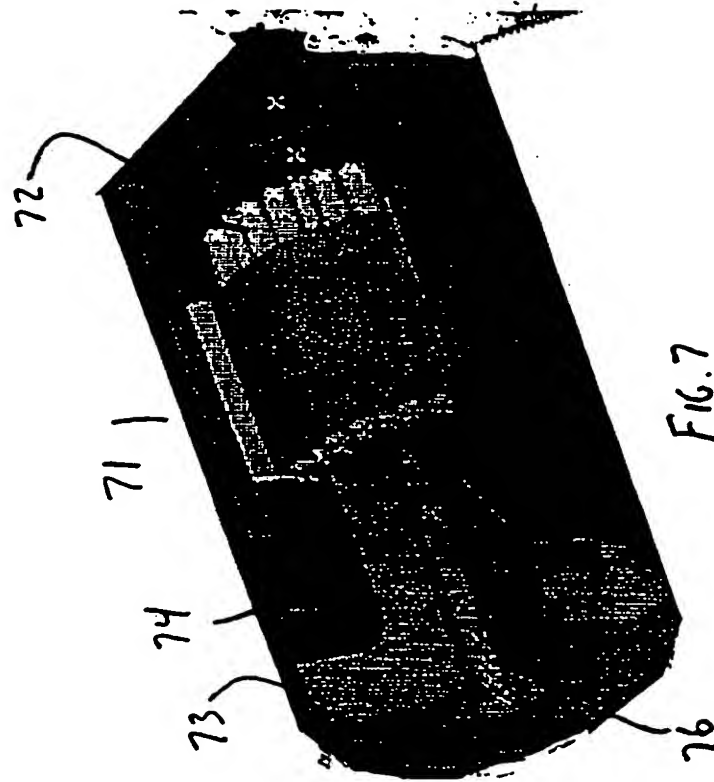
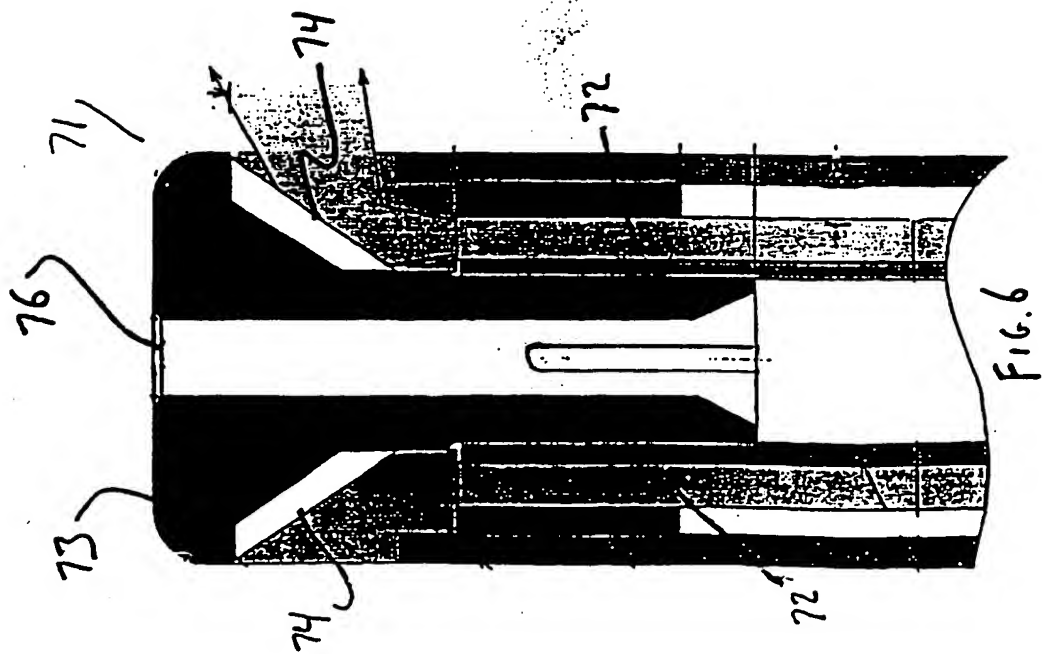




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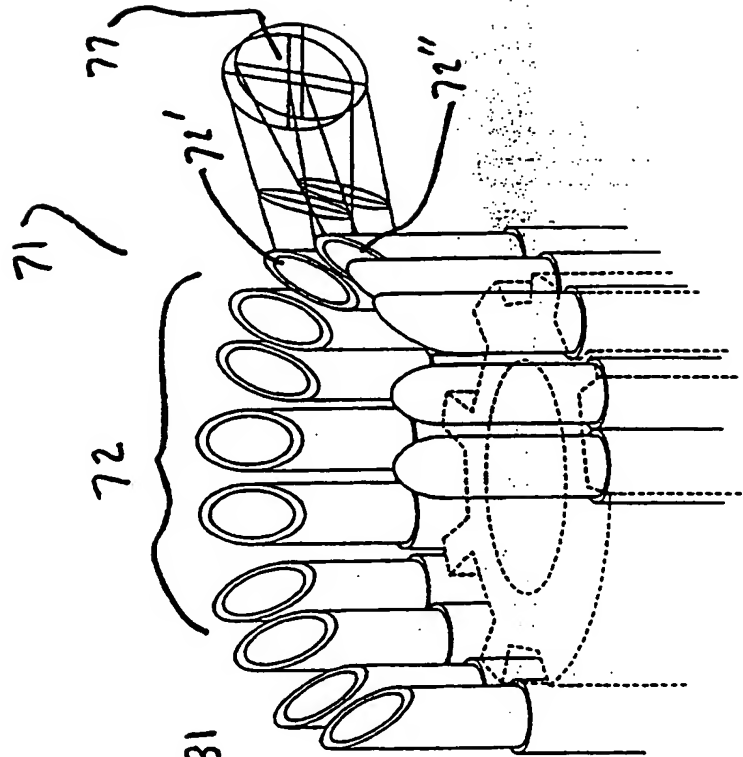


FIG. 9

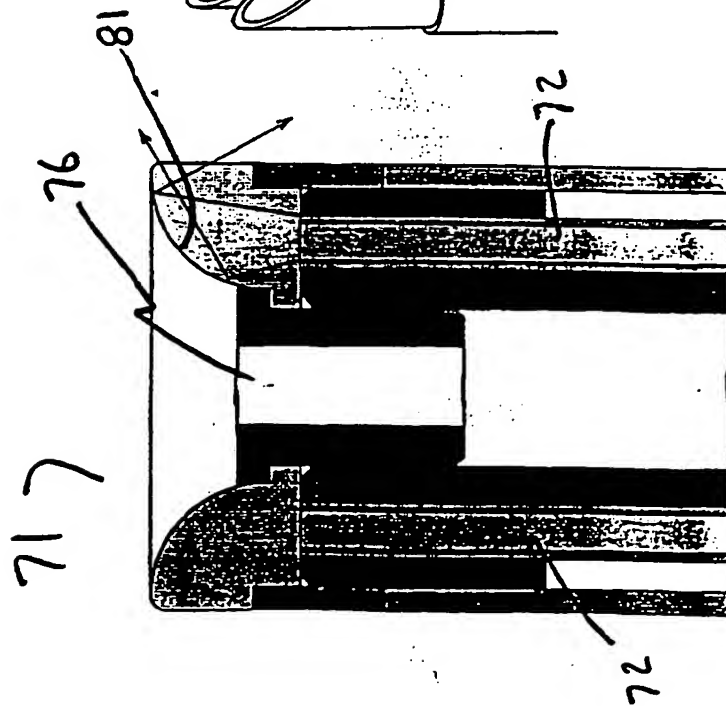


FIG. 8

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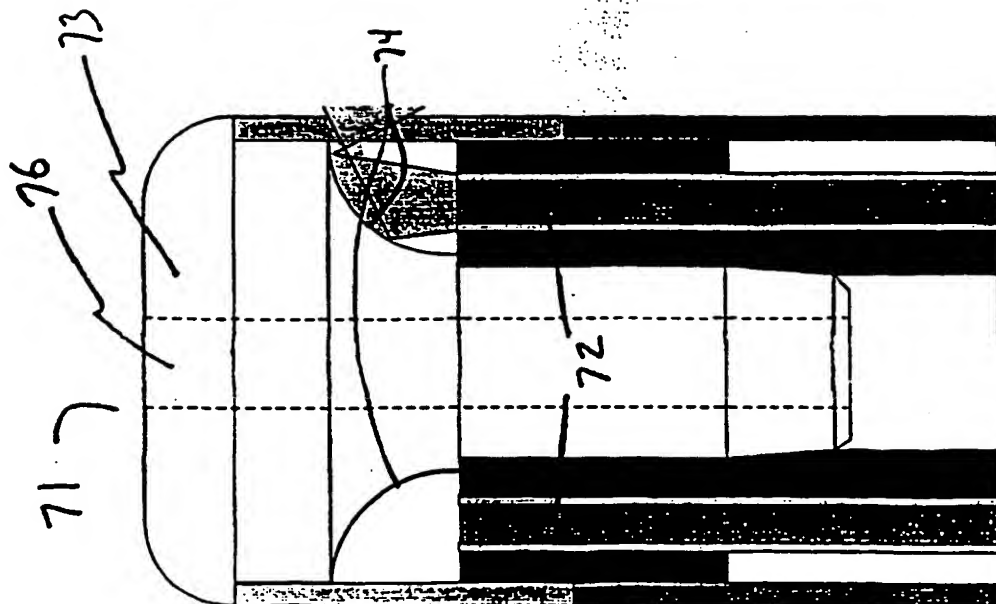


FIG. 10

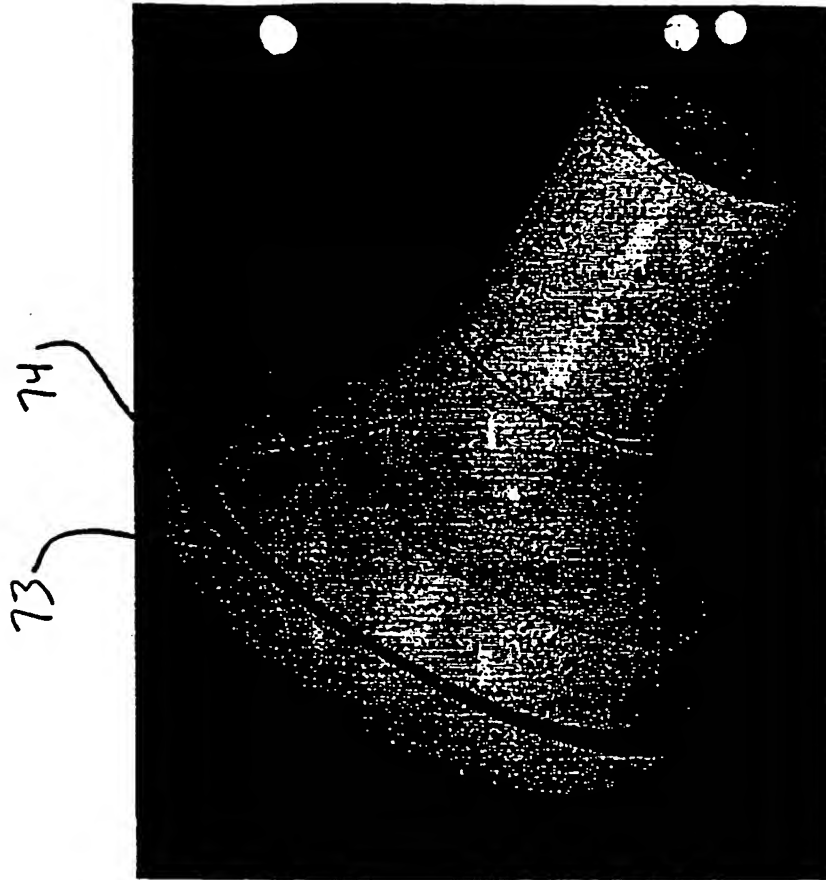
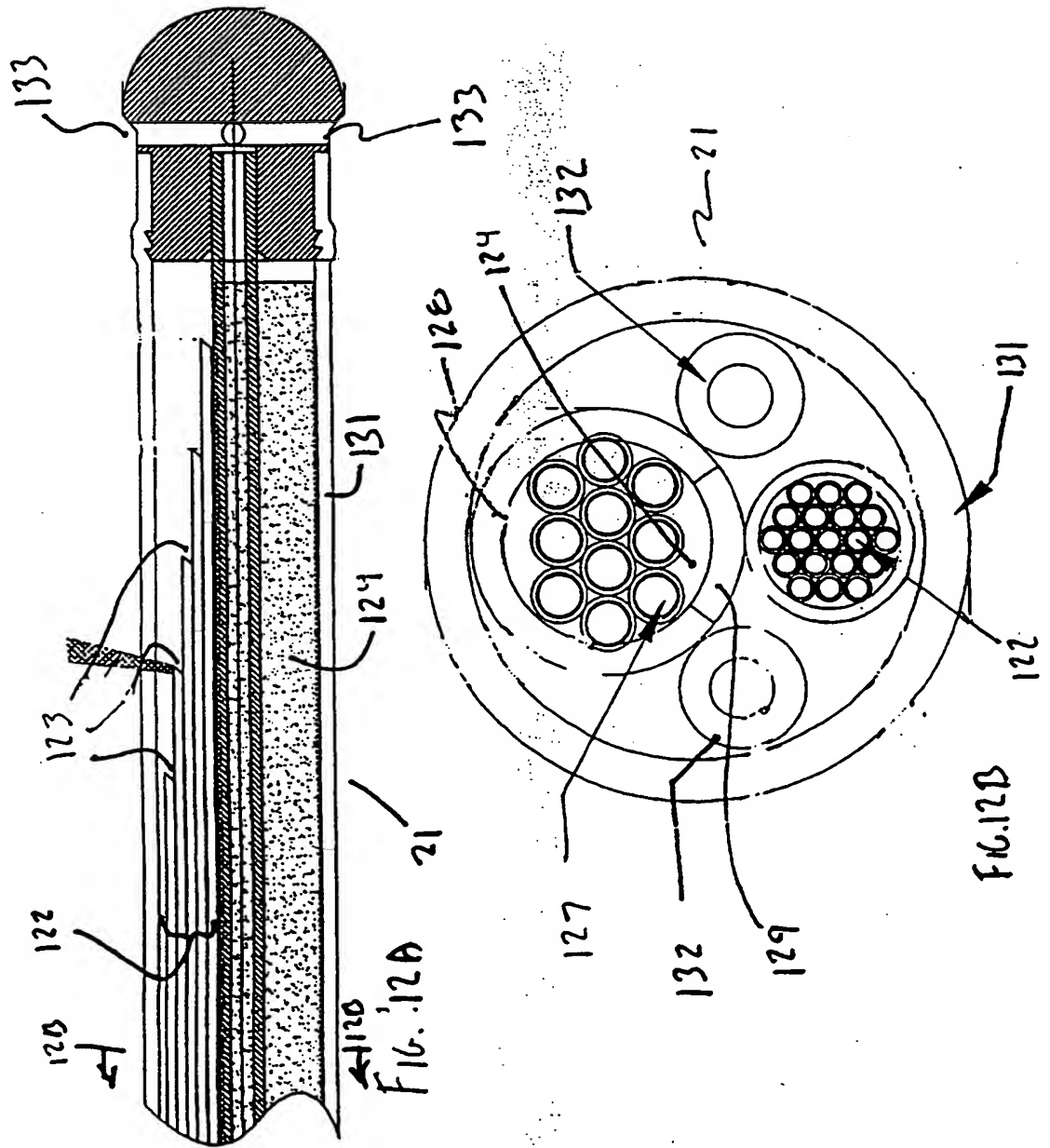
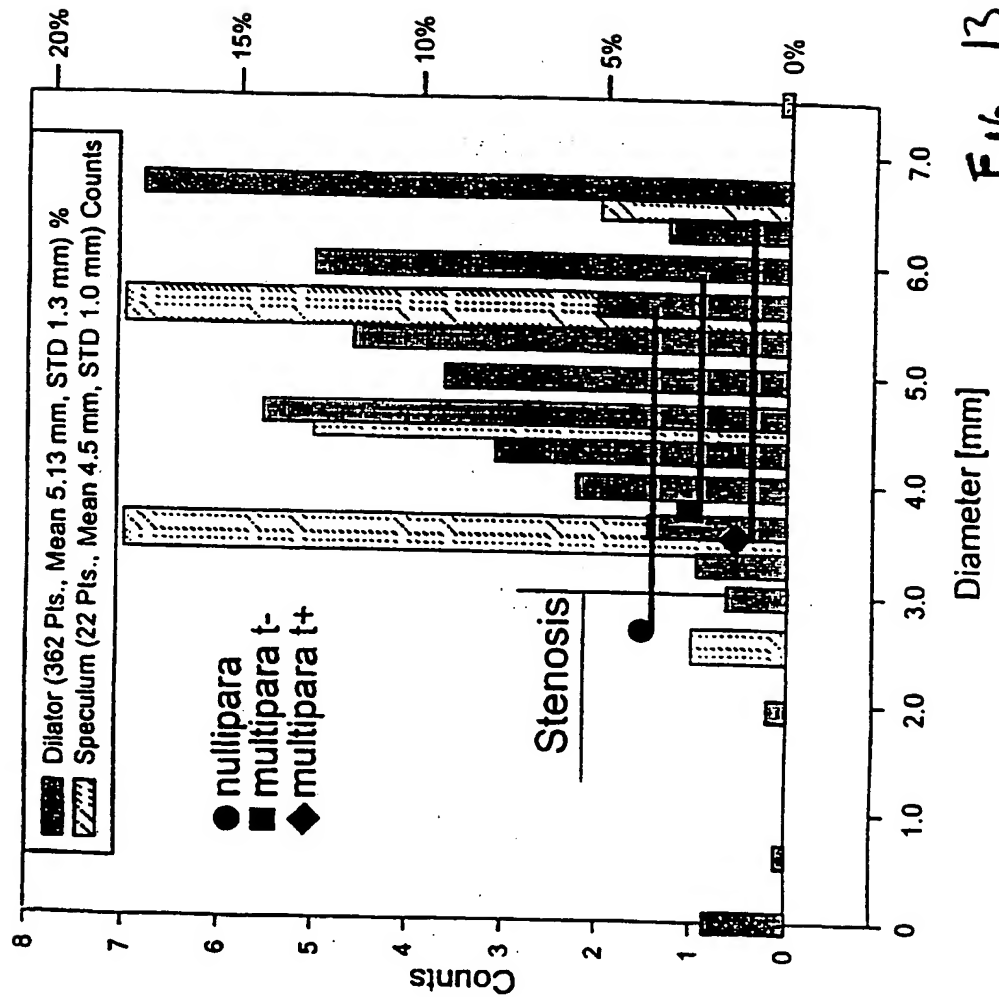


FIG. 11

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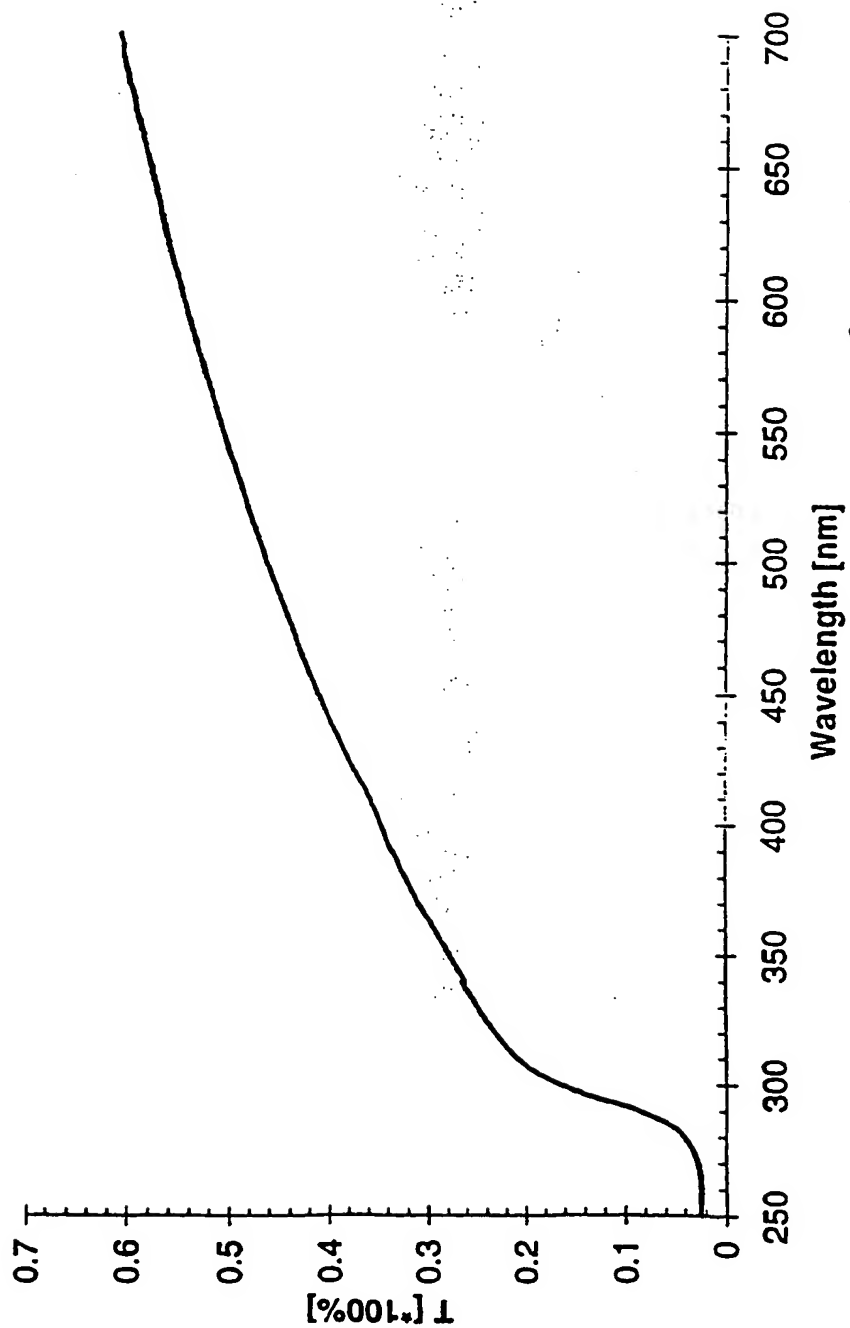


FIG. 14

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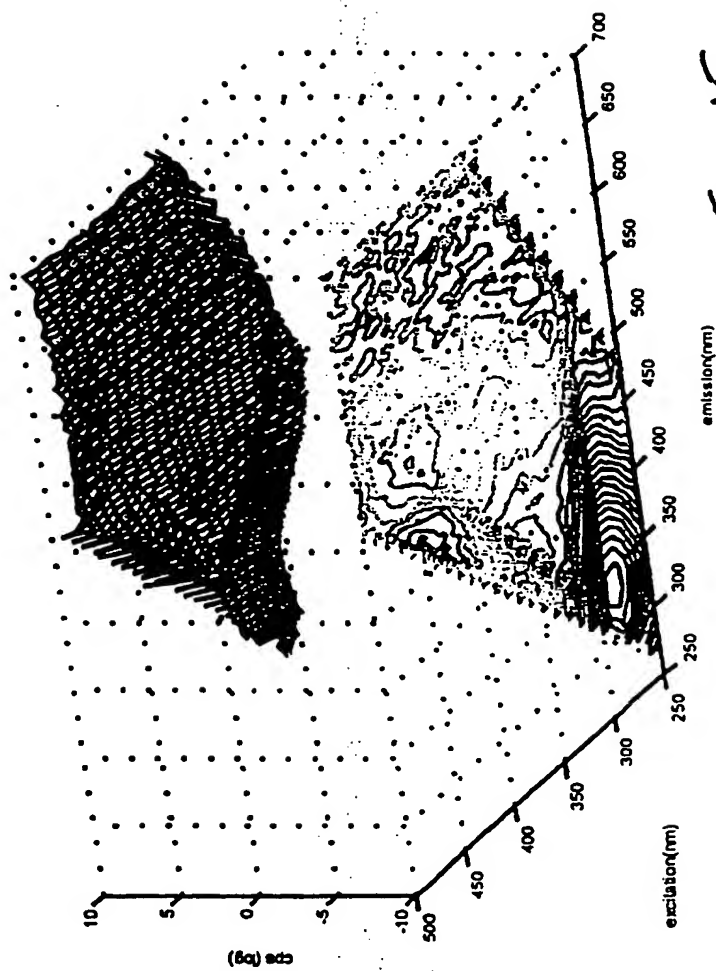


FIG. 15

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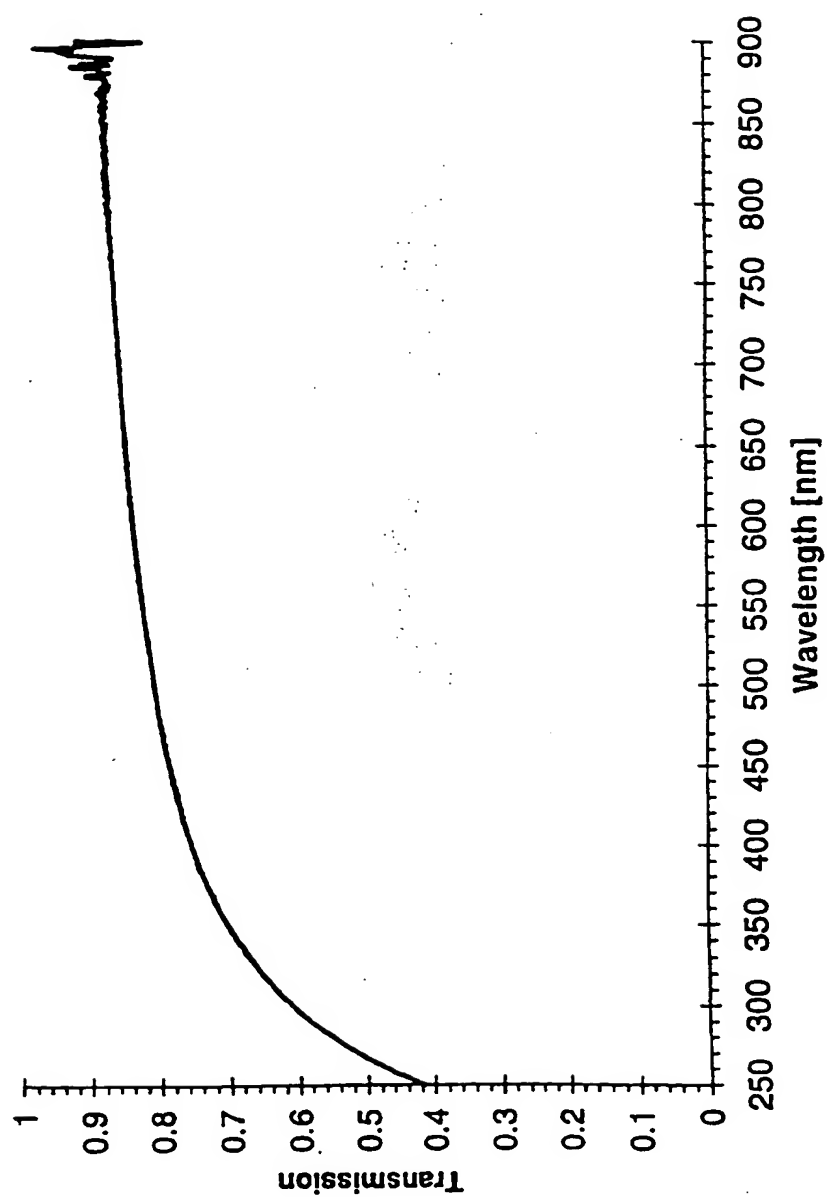
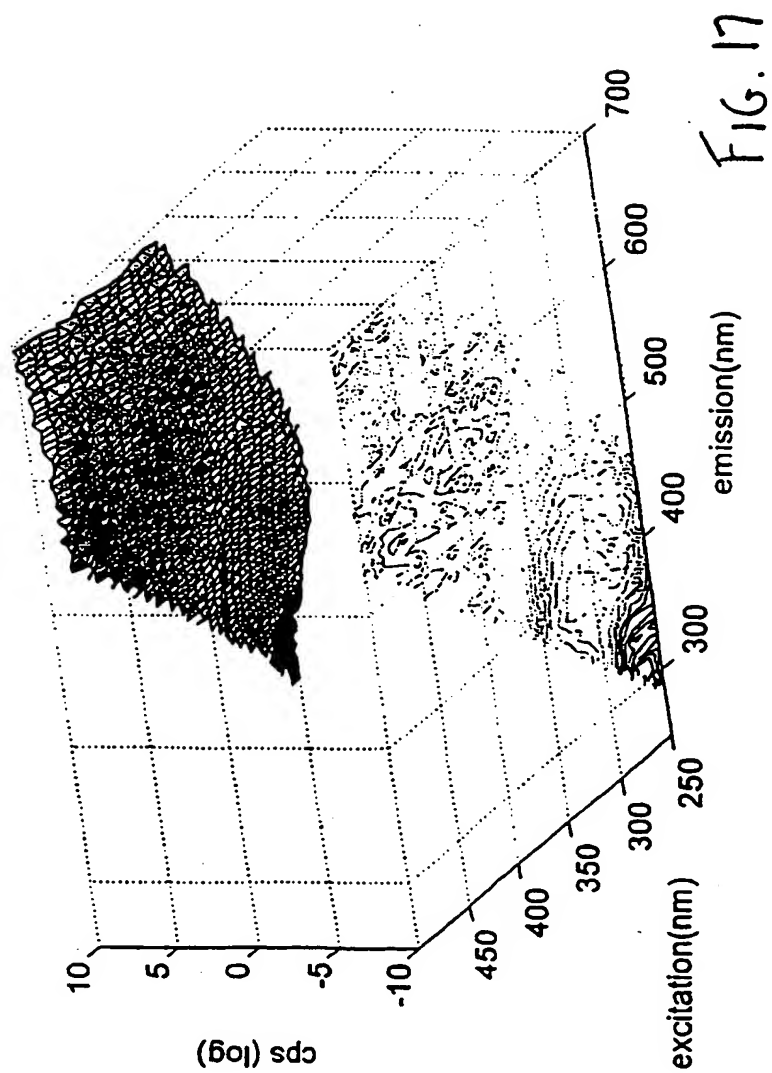


FIG. 16

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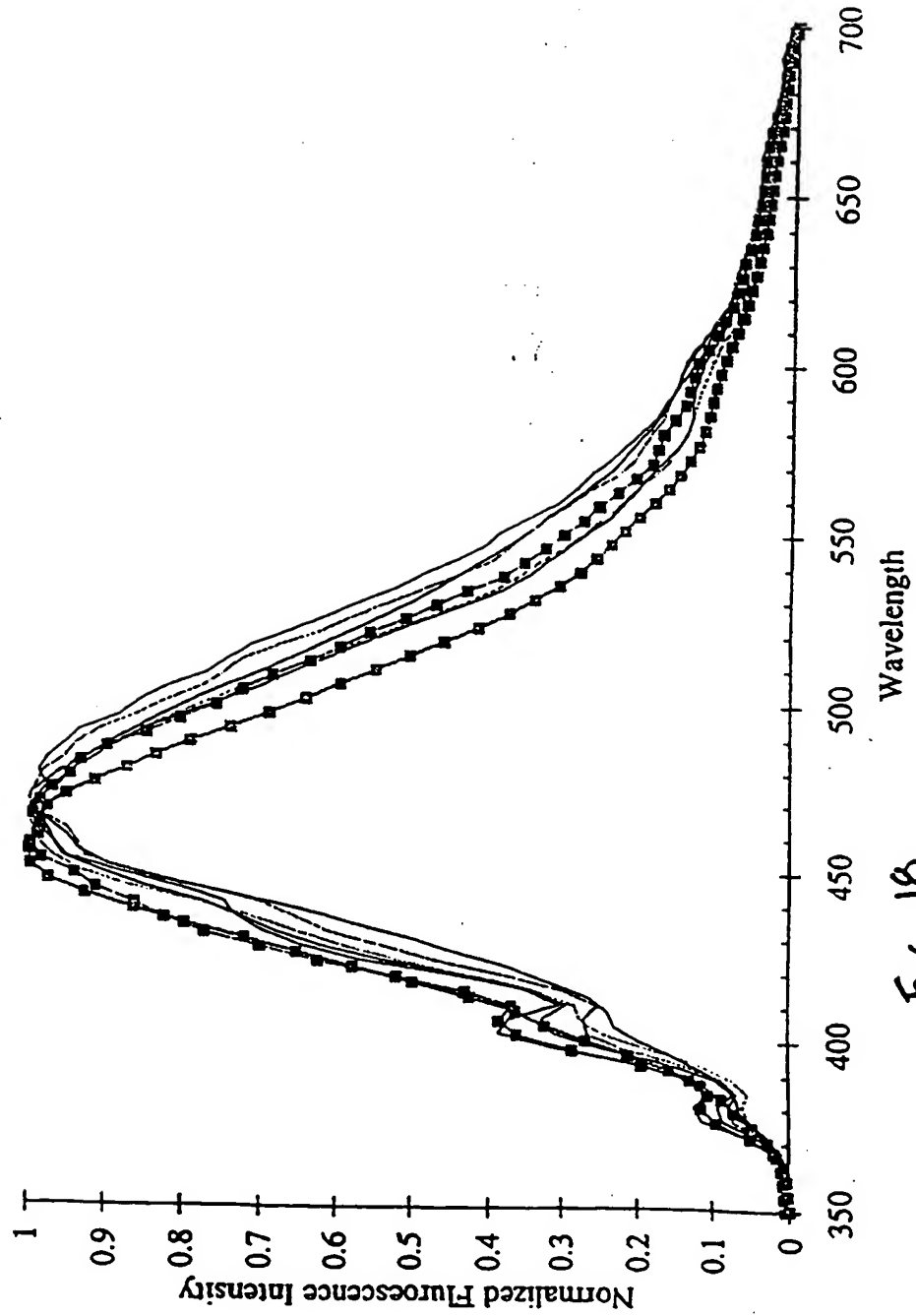


FIG. 18

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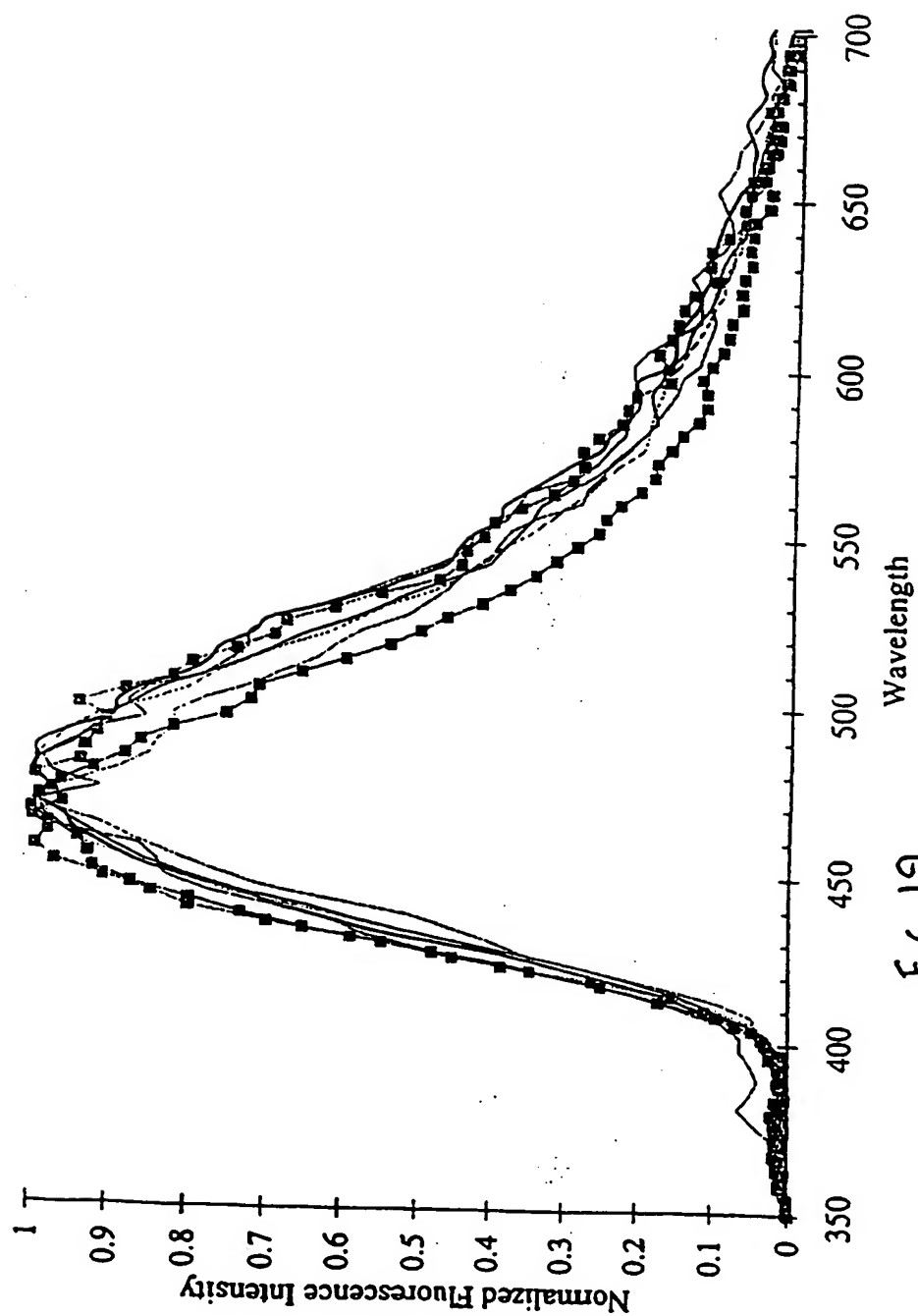


Fig. 19

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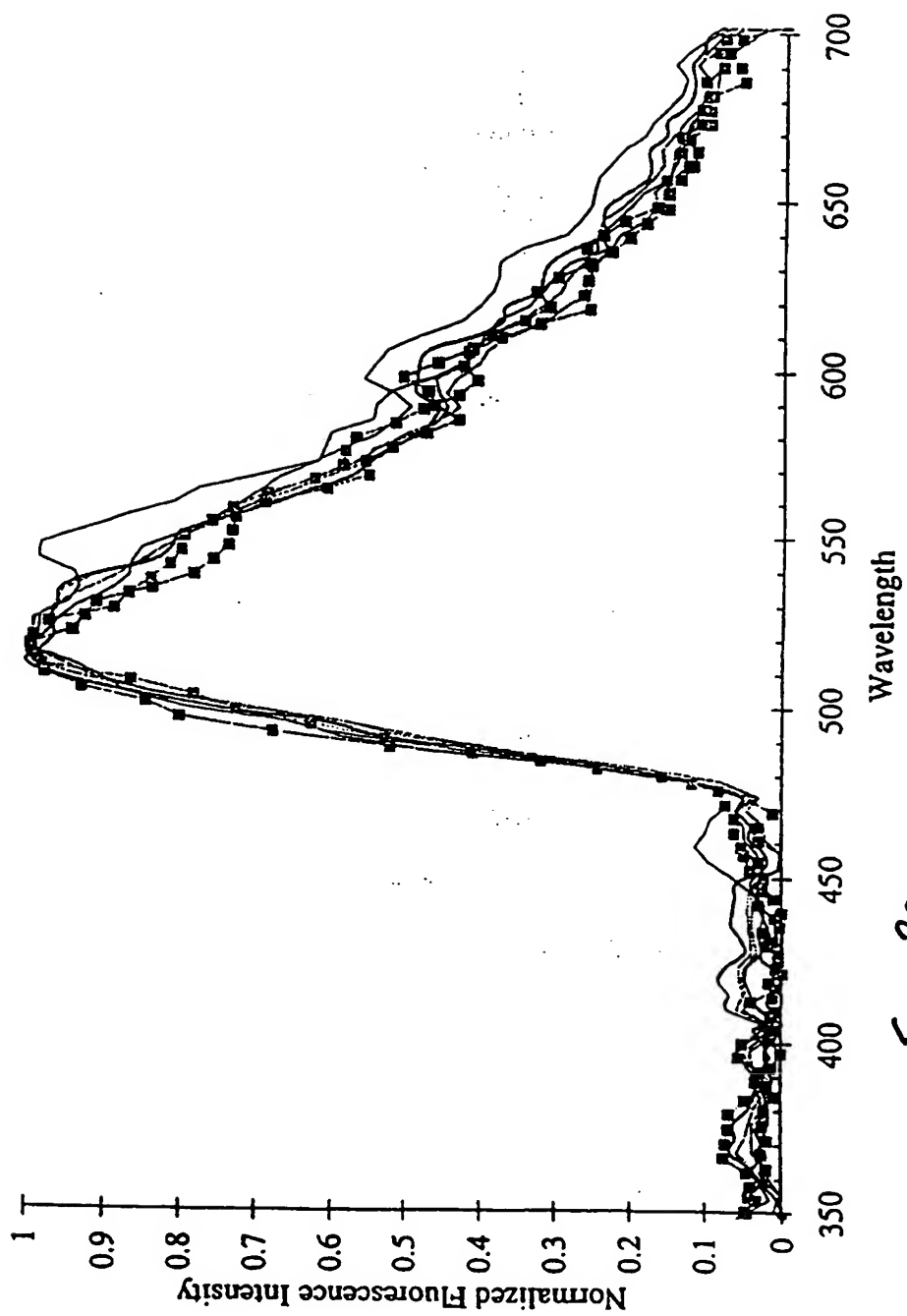


FIG. 20

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/US 97/13300

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 26168 A (UNIVERSITY OF TEXAS) 24 November 1994	1,7,10
X	see page 5, line 35 - page 7, line 31 see page 12, line 10 - page 13, line 20 see page 14, line 9 - page 16, line 13 ---	11,14
X	US 5 450 857 A (R.E. GARFIELD) 19 September 1995	1,7,8
A	see column 2, line 49 - column 3, line 44 see column 4, line 50 - column 5, line 15 see column 8, line 12 - line 28 see column 9, line 1 - line 16 ---	5,14
X	US 5 456 260 A (N. KOLLIAS) 10 October 1995	14
A	see column 1, line 64 - column 2, line 32 see column 3, line 23 - column 5, line 46 ---	1,7,9,10
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 96 28084 A (UNIVERSITY OF TEXAS) 19 September 1996 see page 2, line 34 - page 4, line 23 see page 9, line 12 - page 11, line 13 -----	1,2,7-14
A	A. MAHADEVAN ET AL.: "Optical Techniques for the Diagnosis of Cervical Precancers" SPIE, vol. 2388, 1995, pages 110-120, XP002042626 see page 110, line 1 - page 112, line 12 -----	1,2,7-9, 11-13
A	WO 96 02184 A (WASHINGTON RESEARCH FOUNDATION) 1 February 1996 see page 3, line 30 - line 36 see page 6, line 26 - page 7, line 13 see page 11, line 13 - page 12, line 13 -----	1,3,5,14

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No

PCT/US 97/13300

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9426168 A	24-11-94	US 5421339 A AU 6946894 A CA 2162922 A EP 0702526 A JP 8511179 T US 5623932 A	06-06-95 12-12-94 24-11-94 27-03-96 26-11-96 29-04-97
US 5450857 A	19-09-95	AU 2550595 A CA 2190599 A EP 0759723 A WO 9531929 A	18-12-95 30-11-95 05-03-97 30-11-95
US 5456260 A	10-10-95	NONE	
WO 9628084 A	19-09-96	CA 2190374 A EP 0765134 A	19-09-96 02-04-97
WO 9602184 A	01-02-96	CA 2195075 A	01-02-96

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